The influence of distal environment on peripheral nerve regeneration across a gap

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Summary

Adult mice were divided into groups, all of which had the right sciatic nerve cut. In some groups, both the proximal and distal stumps were put into a plastic tube after various manipulations, while in other groups one or other stump was left out.

Observations following survival periods of between 15 and 60 days showed that successful regeneration took place only in animals in which both proximal and distal stumps were present in the plastic tube and providing the cut ends of both stumps were facing each other. If the distal stumps were excluded from the tube or bent through 180°, regeneration occurred initially, but was eventually ineffective. If the distal stump alone was inserted in the tube, a proliferation of Schwann cells, fibroblasts and regularly aligned collagen occurred.

These findings support the hypothesis that the distal stump is essential for successful regeneration, but that in order to enhance it, the peripheral segment must be perfectly aligned with the proximal stump within the tube. Reasons for the necessity of such an alignment are discussed.

Introduction

It is well known that peripheral nerves are capable of regeneration (Cajal, 1928; Guth, 1956) not only after crush injury but also after transection, whether the two stumps have been previously sutured (Behrmann & Acland, 1981) or left unrepairned (Jurecka et al., 1975). The early stages of the regenerative process after transection have been investigated by Friede & Bischhausen (1980). In the proximal stump of the severed nerve, they described axonal swelling and sprouting, hypertrophy of Schwann cells and the presence of migratory cells outside the fibres or beneath their basal lamina.

Successful regeneration has recently been observed using a model in which severed nerve stumps separated by a gap were enclosed within silicon chambers (Lundborg et al., 1982a, b), polyglyactin (Molander et al., 1982) or semipermeable acrylic tubes (Uzman & Villegas, 1983). Lundborg et al. (1982b) observed that under these conditions axons...
could grow across gaps of up to 10 mm, but that regeneration failed when the gaps were wider or when the distal stump was not inserted into the tube. They emphasized the importance of the presence of the distal stump for the success of the regenerative process, a concept which received additional support from the work of Politis et al. (1982).

While the existence of a factor which promotes the regeneration seems well established, its nature is still unknown. The present study, which describes the process of regeneration of transected sciatic nerves in plastic tubes, is intended to be a contribution to the understanding of the mechanisms involved in the successful regeneration of peripheral nerves in mammals.

Materials and methods

Balb/c mice of both sexes from 3 to 5 months old, weighing 22–25 g, were used in this study. They were divided into six groups, each of which underwent the following procedure (Fig. 1) under intraperitoneal pentobarbitone anaesthesia.

**Proximo-distal group (P-D)**
The skin and the muscles of the postero-lateral aspect of the right thigh were incised longitudinally and the sciatic nerve exposed, mobilized and cut at the midpoint between the sciatic notch and the popliteal fossa. The two stumps were allowed to retract for 2 min and the gap was subsequently increased to 5 mm by trimming the ends of the stump or by retracting them. A ribbon of plastic film (Cling Film PVC 50 thou) was wrapped around the stumps creating a tube containing a short segment of severed nerve at each end. No sutures were made between the cut ends of the nerve and the tube to anchor the stumps which were kept in place by surface tension. The muscles and the skin were sutured taking care to maintain the gap between the stumps, and the animals were allowed to survive for lengths of time varying from 15 to 60 days.

The remaining groups of mice were subjected to the same procedures, but with the following modifications:

**Proximo-open group (P-O)**
The distal stump was removed, or was left lying parallel to the tube but external to it.

**Proximo-disconnected group (P-Dx)**
The distal stump was inserted into the tube but was disconnected from the periphery by severing its distal divisions at the level of the popliteal fossa.

**Proximo-inverted group (P-I)**
The distal stump was disconnected from the periphery and turned through 180°; in this way the original distal end of the nerve (which had become nearer to the tube) was introduced into the distal end of the tube, while the proximal end was left free in the popliteal fossa.

**Proximo-bent group (P-B)**
The distal stump was left in the tube as in A, but its cut end was bent through 180° so that it was directed towards the distal opening of the tube. In this group care was taken to keep the same distance between the two stumps by slightly pulling the proximal end towards the distal.